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Review

Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis

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Summary. According to recent prospective studies, hypofibrinolysis due to elevated plasma plasminogen activator inhibitor 1 levels appears to be an independent risk factor for myocardial reinfarction in men, and hyperinsulinaemia, a major indicator of insulin resistance is considered as a risk factor for coronary disease. It has recently been shown that insulin resistance is accompanied by an increased plasma plasminogen activator inhibitor 1 concentration: A significant correlation coefficient was demonstrated between plasminogen activator inhibitor 1 and fasting plasma insulin in the normal population, in obese subjects, in Type 2 (non-insulin-dependent) diabetic patients and in angina pectoris. Attempts to decrease insulin resistance such as fasting, diet, or administration of an oral anti-diabetic drug such as Metformin induced a parallel decrease in plasma insulin and plasminogen activator inhibitor 1 levels. This inhibitor is produced by endothelial cells and by hepatocytes in culture. Plasminogen activator inhibitor 1 synthesis by hepatocytes in culture was stimulated by an increasing insulin concentration, or low density lipoproteins, whereas the endothelial cell synthesis was stimulated by very low density lipoproteins especially when they were obtained from hypertriglyceridaemic patients. Therefore, a direct effect of insulin or lipoprotein changes on the cells which synthesize plasminogen activator inhibitor 1 could be responsible for its increased plasma concentration in insulin resistance states. The increase in plasma plasminogen activator inhibitor 1 levels linked to hyperinsulinaemia is a tempting partial explanation for the association between insulin resistance and coronary disease.

Key words: Plasminogen activator inhibitor 1, fibrinolysis, atherosclerosis, arterial thrombosis, insulin, insulin resistance, low density lipoprotein, hepatocytes, endothelial cells.

Clinical manifestations of coronary artery disease result from the progressive development of atherosclerotic plaque and subsequent thrombus formation. The role of arterial fibrin deposit in the occlusion of coronary arteries that leads to myocardial infarction is obvious. More subtly, however, fibrin deposit could play a role in the development of atherosclerotic lesions possibly as an initiating factor of endothelial cell injury. Fibrin is a consistent component of human atherosclerotic plaque and it may contribute to plaque growth by stimulation of cell proliferation and by the binding and accumulation of low density lipoprotein [1–4].

Since it leads to the decreased removal of fibrin deposit, hypofibrinolysis would be a prime candidate for a role in the initiation and development of atherothrombosis. Recent findings showed that deficient fibrinolysis is mostly due to the increase in plasma plasminogen activator inhibitor 1 (PAI-1) [5], (Fig. 1). In recent years several studies have demonstrated high plasma PAI-1 in patients with coronary artery disease [6–15] and this appears to be an independent risk factor for myocardial reinfarction within 3 years in men under 45 years-of-age [16–18]. To date no correlation has been demonstrated between PAI-1 levels and the extension of coronary lesions in cross-sectional studies [6, 8, 10, 13, 19]. However, in a recent prospective study, some relationship was observed in patients presenting previous myocardial infarction who were subjected to a glucose tolerance test 3 to 6 months after the acute event and reangiographically assessed 4 to 7 years after the first catheterisation. PAI-1 level was the best predictor of disease progression of coronary atherosclerosis in patients with glucose intolerance [20].

Hyperinsulinaemia is a major indicator of insulin resistance which is associated with several disorders. Grouped under the term "syndrome X" [21], these abnormalities include the following: impaired glucose tolerance, increased blood pressure, modifications of the lipoprotein pattern with increased VLDL triglyceride and decreased HDL cholesterol. This syndrome is mainly observed in subjects with excessive body weight and fat dis-

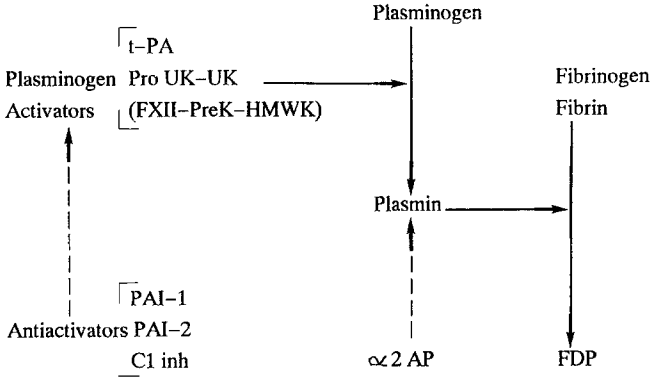

Fig. 1. The fibrinolytic system. t-PA: Tissue-type plasminogen activator, UK: Urokinase, FXII: Factor XII, Pre K: Prekallicrein, HMWK: High molecular weight kininogen, PAI: Plasminogen activator inhibitor, CI inh: CI inhibitor, α2 AP: α2-antiplasmin, FDP: Fibrinogen degradation products

tribution predominant at the upper part of the body [22, 23]. Hyperinsulinaemia is considered as a risk factor for myocardial infarction [24, 25] according to three prospective studies [26–28].

Our group has shown that insulin resistance is accompanied by increased plasma PAI-1 concentration [14, 29–32]. A tempting implication of this finding is that hypofibrinolysis due to a high PAI-1 level contributes to the pathogenic role of the insulin resistant state in coronary artery disease. This paper discusses the relationship between insulin resistance and plasma PAI-1 concentration.

Increased plasma PAI-1 concentration and insulin resistance. Cross-sectional studies

More than 20 years ago hypofibrinolysis was shown to be associated with obesity [33–36], hypertriglyceridaemia [37, 38] and diabetes [39, 40]. This defect has recently been attributed to the increased plasma concentration of PAI-1 accompanying these disorders: Several studies have shown that a very significant correlation exists between PAI-1 concentration and body mass index [14, 29–32, 41–48] waist-to-hip circumference ratio which estimates body fat distribution [31, 43, 46–48] or triglyceride level [8, 16, 18, 30, 43, 47, 49–51]. Increased PAI-1 levels have also been reported in non-insulin-dependent diabetic subjects but insulin-dependent diabetic patients had a nearly normal level of PAI-1 [32, 41, 44, 52, 53]. PAI-1 was also higher in untreated mildly hypertensive men compared to control subjects [51], and PAI activity was correlated with systolic blood pressure in angina pectoris [14].

The relationship between PAI-1 and fasting insulin levels was first demonstrated by our group [29] in normal subjects with a wide range of body weight. We have also observed this relationship in several groups of subjects including non-diabetic obese women [30, 31], Type 2 diabetic patients [32] and patients with angina pectoris [14] (Table 1). Similar observations have been reported by Legnani et al. in obese children [42], by Sundell et al. in subjects fed a low carbohydrate diet [43] and, by Landin et al. in obese women [48], and in non-obese hypertensive men [51].

In all of our studies the correlation coefficient between PAI-1 and insulin was around 0.6 ($p < 0.001$) (Table 1). As previously mentioned the PAI-1 concentration was also correlated with body mass index [29–31], waist-to-hip circumference ratio [31], plasma triglyceride level [14, 31], or apolipoprotein B [32]. Multiple regression analysis has shown that in most cases these correlations disappeared after adjustment for insulin [14, 29, 31, 32]. However, a weak relationship subsisted between triglyceride and PAI-1 in pre-menopausal obese women [31]. These findings suggest that the link between plasma insulin and PAI-1 levels is independent of the other risk factors and that the relationship between PAI-1 level on the one hand and body mass index, waist-to-hip circumference ratio and perhaps triglyceride on the other could depend on the relationship between PAI-1 and insulin, this latter being a witness of the insulin-resistance state.

The association between plasma insulin and PAI-1 levels has not been consistently found [16, 18, 47]. This is probably due to the use of various insulinogenic indexes or parameters of insulin secretion. Plasma insulin may be measured either in the fasting state or after stimulation e.g. glucose load. In non-diabetic subjects, fasting plasma insulin levels are closely related to the degree of insulin resistance [21] while the insulin response to a glucose load depends on both the degree of insulin resistance and the ability of the Beta cell to cope with the stimulus. In a voluntarily heterogenous group of 39 non-diabetic subjects (27 with normal glucose tolerance, 12 with impaired glucose tolerance, ages ranging from 11 to 61 years and body mass index from 17 to 44) we still observed a statistically significant relationship between PAI-1 level and fasting insulin ($r = 0.38$; $p < 0.02$). Conversely the relationship between PAI-1 and other parameters of insulin secretion (insulin sum, insulin increment, insulin area) disappeared or were less significant (insulin at 120 min was correlated with PAI-1) ($r = 0.33$; $p < 0.04$).

In fasting non-diabetic subjects insulin is accompanied in the plasma by insulin precursors, proinsulin, split proinsulin, in a molar ratio of 0.15 [54, 55] and may be as high as 0.25 [56]. These precursors may contaminate the immunoreactive insulin data according to the degree they cross-react with the polyclonal antibody used. However, a contamination of 25% or less (if the cross-reaction is limited as in many insulin assay kits) would have only a limited ef-

Table 1. Coefficient of correlation *(r)* between plasminogen activator inhibitor 1 (PAI-1) and insulinaemia, triglyceride, body mass index, in various groups of subjects

r	PAI-1/ Ins	PAI-1/ TG	PAI-1/ BMI
Normal subjects [29]	0.52[c]	0.16	0.66[c]
Obese non-diabetic women [31]	0.72[c]	0.56[c]	0.50[c]
Type 2 diabetic patients [32]	0.60[c]	0.31	0.31[a]
Angina pectoris [14]	0.59[c]	0.25[a]	0.39[b]

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$

Ins: Insulin, TG: Triglyceride, BMI: Body mass index (weight [kg]/height [m^2])

fect on a close relationship such as the one generally observed between fasting insulin and PAI-1 levels. But in Type 2 diabetic patients, the proportion of precursors is higher and in one study [57] PAI-1 was found to correlate with 32–33 split proinsulin and not with insulin. The interpretation of this finding remains speculative.

Increased plasma PAI-1 concentration and insulin resistance. Intervention studies

The link between insulin resistance and PAI-1 levels observed in cross-sectional studies was confirmed in intervention studies aimed at reducing insulin resistance. The effects of decreasing plasma insulin on PAI-1 levels have been analysed in several studies. It was first shown that a simultaneous drop in insulin, triglyceride and PAI-1 levels was observed in 10 obese women after a 24 h fasting period [29]. The oral anti-diabetic drug Metformin which is known to have a hypoglycaemic effect by improving sensitivity to insulin [58] was shown several years ago to enhance fibrinolytic activity [59]. This effect could be explained by a decrease in PAI-1 level [60]. In a 15 day double-blind placebo study using Metformin (1.7 g/day) in non-diabetic obese women, we observed a decrease in fasting plasma insulin, triglyceride as well as PAI-1 levels in the treated group but not in the placebo group [60].

Since weight loss improves the insulin resistance of obese subjects [61], it should also lead to a decrease in PAI-1. We observed the evolution of PAI-1 over a several-month period in a 21-year-old obese woman who had suffered cerebral venous thrombosis, after losing weight on a low-caloric diet, the normalization of plasma insulin and triglyceride was accompanied by the normalization of PAI-1 levels [50]. Similar changes in PAI-1 have been reported after 2 weeks [45], 6 weeks [62] or 13 weeks [63] hypocaloric diet. These findings are in agreement with those of Andersen [38] and Simpson [64] who observed increased fibrinolytic activity in high risk male coronary patients after diet and clofibrate therapy aimed at normalizing triglyceride level.

The beneficial effect of physical exercise on insulin resistance is well-known [65], and it has been shown that physical training also improves fibrinolytic activity [64, 66]. It was recently demonstrated that the increase in fibrinolytic activity after physical training was due to a decrease in PAI-1 levels [67, 68].

Attemps at explaining the increased plasma PAI-1 levels in insulin resistance

The existence of a significant correlation between plasma PAI-1 and insulin concentration, the simultaneous variations of these two parameters in intervention studies aimed at reducing the insulin level suggest that insulin may act directly or indirectly on the cells which synthesize PAI-1 i.e. endothelial cells and hepatocytes [69, 70].

Our group showed [71] that adding insulin to umbilical vein endothelial cell culture had no effect on PAI-1 synthesis. In contrast, insulin at 0.6^{-9} molar concentration induced after 24 h a two-fold increase in PAI-1 production by the hepatocyte cell line HepG2. This concentration of insulin is similar to the one found in the portal vein after meals. This effect seems to be specific for PAI-1 since the synthesis of other proteins of hepatic origin, e.g. fibrinogen or α2-antiplasmin was not modified. This insulin effect was reproduced in human hepatocytes in culture and was accompanied by an increase in PAI-1 mRNA [72]. Similar results were obtained by adding insulin-like growth factor 1 (IGF-1) to HepG2 cells [73]. The fact that the DNA content was not modified after insulin or the addition of IGF-1 to the cells excludes a proliferative effect at the concentrations used.

The stimulating effect of insulin on PAI-1 synthesis by HepG2 cells was abolished when the anti-diabetic drug Metformin was added to the cell cultures at the same time as insulin (unpublished data). This inhibitory effect of Metformin was dose-dependent being significant at 10^{-6} molar concentration. Therefore, the insulin added to hepatocytes in culture stimulated their production of PAI-1 and this effect was abolished by Metformin.

In vivo this direct effect on PAI-1 production by the liver was not observed after acute administration of insulin [74, 75]. Even with a hyperinsulinaemic glucose clamp for 24 h [76] we failed to increase PAI-1 levels or to break down the well-known circadian rhythm of PAI-1 levels which shows a peak in the early morning and a nadir in the afternoon [77]. Medvescek et al. [78] studied the effect of the acute rise in endogenous insulin following an oral glucose load. It induced a discrete transitory increase in PAI-1 levels one hour after the peak of insulinaemia. This effect did not mask the diurnal decline in PAI-1 and was similar in lean and obese subjects.

PAI-1 concentration in plasma is therefore not directly related to insulin level at least under acute conditions which are not accompanied by insulin resistance or sustained non-esterified fatty acid flux. Thus, a discrepancy exists between in vitro and in vivo data concerning direct effects of insulin on PAI-1 levels. One hypothesis to explain this discrepancy is that the cells in culture present insulin resistance, which mimics the in vivo conditions of insulin resistance. This hypothesis is supported by the fact that HepG2 cells possess defective insulin receptors on the membrane surface [79]. Furthermore, adding Metformin along with insulin to insulin resistant rat hepatocytes restored normal lipogenesis [80].

The mechanism by which insulin influences the release of PAI-1 may be either direct or indirect through modification in plasma lipoproteins. Given its effects on hepatic synthesis of VLDL triglyceride [81], insulin could also act indirectly by quantitative or qualitative changes of lipoproteins. Interestingly Stiko-Rahm et al. [82] showed that purified VLDL stimulate PAI-1 secretion by endothelial cells from umbilical vein and that VLDL from hypertriglyceridaemic subjects were more potent than VLDL from normotriglyceridaemic patients, the large particle subfraction was observed to produce the greater PAI-1 release. The stimulating effect of VLDL was dependent on binding of the lipoprotein particles to apolipoprotein B/E receptors of the cells as the effect was abolished in presence of an antibody against the apolipoprotein B/E receptors.

Another argument for the role of circulating lipid levels in regulating PAI-1 concentration is that we have shown strong stimulation of the hepatocyte synthesis of PAI-1 by normal non-oxidized LDL [83], this effect being dependent of the binding of LDL to the apolipoprotein B/E receptors.

Taken together these in vitro data strongly indicate that PAI-1 synthesis by both hepatocytes and endothelial cells could be affected by high insulin concentration and lipoprotein abnormalities in patients with insulin-resistance states.

Conclusion

Insulin resistance may increase the risk of coronary disease by promoting the formation of atheromatous lesions and development of thrombosis. A defect in the fibrinolytic system could be responsible for the initiation and the late complications of atherothrombosis. The increase in PAI-1 concentration linked to hyperinsulinaemia is a tempting partial explanation for the association between insulin resistance and coronary disease.

Normalization of PAI-1 by reducing insulin resistance could slow down atherothrombosis and prevent coronary disease. Further epidemiologic and intervention data are needed to confirm this hypothesis.

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